APPENDIX C

Peabody ENERGY

Memorandum

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Technical Memorandum on Whole Effluent Toxicity (WET) Testing

Whole Effluent Toxicity (WET) Testing at Midwest mine sites is inappropriate based on the recognition that WET testing is not accurate in the context of mining operations and the streams that are typically present at these operations. Consistent with conditions at Bear Run, many of the water bodies confronted at mine sites in the Illinois Basin are ephemeral or intermittent streams. Because of the sporadic flow, these streams typically do not support obligate aquatic organisms and, accordingly, acute tests are overprotective and unreliable. A chronic WET test in an intermittent stream is overprotective of limited aquatic life with non-continuous wastewater discharges. Daphnia magna and fathead minnows are the only appropriate chronic WET test species when receiving waters exhibit naturally elevated salinity or dissolved solids conditions and discharges are continuous and total suspended solids (TSS) discharge limits are at or above 35 mg/L. For these reasons, EPA WET testing guidance allows for state exemptions from chronic WET testing requirements for zero/low flow conditions (USEPA draft 2004, National Whole Effluent Toxicity (WET) Implementation Guidance Under the NPDES Program, Office of Wastewater Management, EPA Doc. 832-B-04-003 released December 28, 2004). Accordingly WET testing is an inappropriate means to evaluate discharges from Bear Run.

WET test species Ceriodaphnia dubia is not natively present at the site (Bioassessment Conducted for the Bear Run Mine Amendment 5 404 Permit). Not all species show the same resistivity to effluent, both to individual and combined contaminants in effluent, as they differ in the ways they respond to contaminant exposure. How the species sequester or eliminate (depurations) exposure to the contaminant, whether or not the species has a prior history of exposure (acclimation) or adapted sensitivity to the contaminant, and its type of exposure and avoidance capabilities are all important factors to be considered (Chapman, 2000). Differences in tolerance levels can be large even amongst WET test species. Differences in the maximum acceptable toxicant concentrations (range between NOEC and lowest observed effect concentration) of about an order of magnitude have been found between Daphnia magana (56-75%), Daphnia pulex (1-10%), and Ceriodaphnia dubia (25-56%) (Chapman, 2000; Chapman et al., 1994). Similar differences have been found with exposure to individual and inorganic chemicals. Thus the use of a single toxicity value elucidated from a WET test conducted on a single non native species is likely non representative of the native aquatic assemblage and should not be used as a bright line regulation.

The laboratory is a controlled environment that eliminates many of the abiotic (climate, temperature, general environmental quality) and biotic (species, life stage, sex and reproductive status, nutritional and disease status, competition and predation) modifying factors that can impact an organism's response to toxicants. WET tests should not be used as an absolute prediction tool for aquatic species response in natural conditions because they do not incorporate relative sensitivities of the

laboratory versus the field, covariates of toxicity (i.e. additive or synergistic effects), differences of exposure routes (food is an exposure route not considered by WET tests), and often use nonindigenous organisms (Chapman, 2000). Not only can sensitivies differ between laboratory cultures and field collected populations but other factors such as size, age, sexual differences, timing to molt, and seasonal differences can also affect the organism's sensitivities (Chapman, 2000; McGee et al., 1998; Rand, 1995). Whole effluent toxicity levels are generally, but not always, overprotective (Chapman, 2000).

WET tests are typically conducted under conservative exposure conditions, where test organisms are exposed to non-normal and worst case dilution conditions. Non normal conditions can result in pre-stress conditions that increase the organism's sensitivity to other stressors. Changes in temperature or background water quality (for instance low dissolved or suspended solids, which allows toxicants to be more bioavailable throughout the water column) can have significant impacts on toxicity results. For example, hardness can skew the results of the toxicity test and may affect the expression of toxicity in the conduct of the test (i.e. the accuracy of the tests at predicting toxicity) (USEPA 1996). Other parameters such as TDS(hardness, salinity, conductivity), turbidity, DO, pH, micronutrients, and bacteria counts can impact test organisms physiology, sensitivity, and biological response, therefore test variability at all levels can be affected by variability in dilution water quality (USEPA, 2000). This has led the EPA, in its published methods manual , to disqualify some WET results when unusual ionic conditions are present, "Adverse effects of low dissolved oxygen (DO) concentrations, high concentrations of suspended and/or dissolved solids, and extremes of pH, alkalinity, or hardness may mask the presence of toxic substances " (USEPA 2002). Because of the possibility of temporary elevated TDS concentrations at some outfalls, the facts presented here would make the use of WET tests at mines unreasonable. This fact was recognized by EPA Region 5 during the Vermillion Grove study.

WET testing is typically related to worst-case dilution conditions rather than the actual receiving stream dilutions (Chapman, 2000). This is especially true in mining environments with intermittent discharge where the first ephemeral stream capable of supporting aquatic assemblages may be a significant distance downstream of the watershed. In addition effluent composition changes over time and discharges from outfalls are intermittent at mining sites. Effects of intermittent exposure to toxicants can be significantly different from effects related to sustained exposure, which is inherent to WET tests. Several cases have shown toxicity from intermittent exposures can result in less toxicity than sustained exposures (Fisher et al., 1994; Hosmer et al, 1998). Differences between sustained and intermittent exposure were recognized prior to the implementation of WET tests (Ingersoll and Winner 1982; Cairns et al., 1981), but have received limited study. WET tests are conducted in the absence of environmental processes, such as photodegradation, sorption and transformation, biodegradation, hydrolysis, and oxidation and reduction that could ameliorate exposure (toxicity) in the wild. WET tests do not account for avoidance strategies or ecological compensation and regulation mechanisms that often allow for species acclamation or adaptation. For example populations of organisms have been shown to evolve resistance to metal contaminants (Klerks and Weis, 1987; Leppanen et al., 1998). WET testing is inappropriate and expensive, especially considering the how unreliable the results may be.

APPENDIX C – EXHIBIT 1

REFRENCES

Carins, J. Jr., Thompson, K.W., Hendricks, A.C. 1981. Effects of fluctuating, sublethal applications of heavy metal solutions upon the gill, ventilator response of bluegills (*Lepomis macrochirus*). EPA-600/S3-81-003. Technical Report. U.S. Environmental Protection Agency, Duluth, MN.

Chapman, P.M. 2000. Whole effluent toxicity testing-usefulness, level of protection, and risk assessment. *Environ. Toxicol. Chem.* 19:3-13.

Chapman, P.M. Paine, M.D., Moran, T., Kierstead T. 1994. Refinery water (intake and effluent) quality: Update of 1970s with 1990s toxicity testing. Environ Toxicol Chem 13:897-909.

Fisher, D.J., Burton, D.T., Yonkos, L.T., Turley, S.D., Turley, B.S., Ziegler, G.P., Zillioux, E.J. 1994. Acute and short-term chronic effects of continuous and intermittent chlorination on *Mysidopsis bahia and Menidia beryllina*. *Environ Toxicol Chem* 13:1525-1534.

Hosmer, A.J. Warren, L.W., Ward, T.J. 1998. Chronic toxicity of pulse-dosed fenoxycarb to *Daphnia magna* exposed to environmentally realistic concentrations. *Environ Toxicol Chem* 17:1860-1866.

Ingersol, C.G., Winner, R.W. 1982. Effect on Daphnia pulex (De Geer) of daily pulse exposures to copper or cadmium. *Environ Toxicol Chem* 1:321-327.

Klerks, P.L., Weis, J.S. 1987. Genetic adaptation to heavy metals in aquatic organisms: A review. *Environ Pollut* 45:173-205.

Lappanen, M.T., Postma, J.F., Groenendijk, D., Kukkonen, J.V.K., Buckert-de Jong, J.C. 1998. Feeding activity of midge larvae (*Chironomus riparius Meigen*) in metal-polluted river sediments. *Ecotoxical Environ Saf* 41:251-257.

McGee, R.L., Wright, D.A., Fisher D.J. 1998. Biotic factors modifying acute toxicity of aqueous cadmium to estuarine amphipod *Leptocheirus plumulosus. Arch Environ Contam Toxicol* 34:34-40.

Rand, G.M. 1995. *Fundamentals of Aquatic Toxicology*, 2nd ed. Taylor and Francis, Washington DC.

U.S. EPA. "Clarifications Regarding Flexibility in 40 CFR Part 136 Whole Effluent Toxicity (WET) Test Methods" Memorandum from Tudor T. Davies, Director Office of Science and Technology to Water Management Division Directors, Regions I-X Environmental Services Division Directors, Regions I-X; April 10, 1996.

U.S. EPA. Short-Term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Water to Freshwater Organisms, Fourth Ed. EPA-821-R-02-013. October, 2002. Section 11.3.2 @ pg. 53

Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the NPDES Program. EPA-833-R-00-003 (June, 2000); p. D-7 (WET-IX Docket #B.12).